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13. ABSTRACT (Maximum 200 Words)
A novel system for studying growth of normal human mammary epithelium in vivo as grafts in athymic nude mice has been developed (Parmar et al., 2002). The key feature of this model is the reconstitution of the epithelial-stromal interactions that occur in the normal human breast. This model has been used to demonstrate the ability of carcinoma associated fibroblasts to cause abnormal growth of normal human mammary epithelium. The renal grafting technique has also been used to study tumor growth and tumor inhibition.

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INTRODUCTION

The main focus of this research is the study of epithelial-stromal interactions that take place in the normal and the tumorigenic mammary gland. To aid in this study we have developed a new model in which organoids from breast reduction mammoplasty are combined with mammary fibroblasts from either mouse or human origin. After growth in collagen under the renal capsule of a female nude mouse, ducts are formed which express appropriate markers for mammary ductal development and respond to hormonal influences (Parmar et al., 2002). This model has also been used to show the effects of carcinoma associated fibroblasts on normal breast epithelium development and the renal grafting method has been used to study inhibition of tumor growth.

BODY:

Technical objective 1: Profile ESX expression in normal developmentally staged human breast epithelium.

Using in situ hybridization, ESX expression was found in the luminal cells of human breast epithelium. An ESX antibody is still not available that is suitable for immunohistochemistry so no further progress has been made with this part of the project. However, I have collaborated on a related project analyzing ESX expression using oligonucleotide microarray analysis technology. This was achieved using an Affymetrix chip U133A and determined ESX (ELF3) mRNA levels in 50 human breast cancer cell lines and 50 human tumors. A manuscript describing this data is currently in review (Neve et al.).

Technical objective 2.1: Confirm that embryonic mammary mesenchyme is capable of inducing a mammary specific pattern of ESX expression.

Embryonic mammary mesenchyme and mammary epithelium develop into phenotypically normal mammary gland when placed under the renal capsule of female nude mice. The developing glands have been removed at various stages throughout the reproductive cycle, formalin-fixed and embedded in paraffin. The blocks are still in storage since an ESX antibody is still not available.

Technical objective 2.2: Testing the correlation between stromal age and tumor induction on epithelial ESX and ErbB2 expression.

Since there is a link between aging and oxidative damage, the passed year has focused more on the mammary glands obtained from SOD2 knockout mice (Melov et al., 1999). Oxidative stress and excess exposure to reactive oxygen species (ROS) have been linked to the initiation and progression of malignancies including breast cancer. Manganese superoxide dismutase (SOD2), as a key mitochondrial enzyme that prevents buildup of intracellular ROS, has been proposed as a tumor suppressing factor. The aim of the present study was to evaluate the potential tumor suppressing activity of SOD2 by assessing mammary gland development in SOD2-null mice that typically die within a week of birth and oxygen inspiration. To investigate the potential mammary gland dysregulating effect of excess intracellular ROS production, mammary gland anlage from newborn female mice with normal or absent SOD2, SOD2+/+ or SOD2-/- (null), were excised and implanted under the renal capsule of normal host female nude mice with/without concurrent estrogen (DES) supplementation. After 30 days in vivo growth, the transplanted glands were excised for wholemount, microscopic and immunohistochemical evaluation. In contrast to the normal growth and development of transplanted SOD2+/+ glands, SOD2-/- glands showed arrested development with reduced ductal outgrowth and branching, and absent ductal lumen. These hypomorphic ductal structures contained dysplastic epithelium with increased Ki-67 labeling, loss of E-cadherin expression, and disorganized p63 and cytokeratin (K)-14 expressing basal and myoepithelial cells. Estrogen treatment (DES x 30 days), while completely downregulating nuclear estrogen receptor (ER) expression, failed to upregulate progesterone receptor (PR) expression or to normalize duct and lobular development. These findings indicate that excess oxidative stress from the absence of SOD2 activity arrests mammary gland development by impairing ductal outgrowth and branching as well as hormonal responsiveness. The resulting dysplastic and immunohistochemical features associated with this arrested development are suggestive of early neoplastic changes.

Technical objective 2.3: Employ ESX-null epithelium to demonstrate its function in mesenchyme-induced mammary gland development.
We are still unable to secure ESX-null mammary gland tissue from the Australian investigators who developed and described this model. Results from the pronuclear injection method to create ESX null mutants also proved unsuccessful. Given that this is now a long-term goal, it is unlikely that this technical objective will be accomplished during the course of my DOD funding.

Technical objective 3: Demonstrate that the abnormal stromal microenvironment provided by CAF perturbs ESX and/or ErbB2 induction in mammary epithelial cells differently from that of heterotypic stromas; in particular, determine if CAF from ErbB2 tumors can produce an exaggerated induction in non-malignant mammary epithelial cells.
This study was carried out using the described method of taking organoids and recombining them with fibroblasts (Parmar et al., 2002). In this case, using fibroblasts from tumor tissue. Work to determine the effects of using carcinoma-associated fibroblasts (CAF) has been done in collaboration with Shanaz Dairkee at the California Pacific Medical Center. Dr. Dairkee is part of the breast cancer SPORE and has provided me with cells and organoids from banked tissue taken from breast cancer patients. Using epithelial cells or organoids peripheral to the breast tissue combined with matched CAF has resulted in abnormal epithelial development since normal ductal morphology was not observed. Immunohistochemistry was done on sections from these recombinations and showed a decrease in the E-Cadherin and laminin expression. Ducts did not express the markers for luminal epithelial cells (K8) or myoepithelial cells (K14) appropriately. ErbB2 expression also appeared elevated compared to recombinations from normal epithelium and fibroblasts. To elaborate on these findings, Dr. Dairkee's lab will be using these recombined grafts to test for genetic alterations in the normal epithelium combined with CAF.
Using the renal graft site, I used MCF-7 cells to test whether tumors could be grown and whether tumor growth could be inhibited. MCF-7 cells are an established epithelial breast cancer cell line. 250,000 cells were placed in collagen then grafted under the renal capsule for one month. In collaboration with Dale Leitman at UCSF this method was used to address the role of ERβ in tumor development. MCF-7 cells were infected in vitro using an adenovirus expressing ERβ and then placed under the renal capsule. Using this method tumor growth was inhibited suggesting a tumor suppressive role for ERβ. This work has been published in cancer research (Paruthiyil S et al., 2004).

KEY RESEARCH ACCOMPLISHMENTS:
Development of a novel method of growing human breast epithelium in vivo
Learnt renal grafting procedure
Grown mouse embryonic mammary buds successfully under the renal capsule
Successful tissue analysis using immunohistochemistry
Publication of 3 manuscripts with 2 in preparation
Invited to speak at prestigious research meetings

REPORTABLE OUTCOMES:
Poster and abstract. UCSF joint breast and prostate meeting. San Francisco 2003.
Poster: San Antonio breast cancer symposium. Dec 2003.

PAPERS IN PRESS AND IN PREPARATION:

Parmar H, Young P, Emerman JT, Neve RM, Dairkee S and Cunha GR. A novel method for growing human breast epithelium in vivo using mouse and human mammary fibroblasts. Endocrinology. 2002 Dec;143(12):4886-96

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Neve, R.M., **Parmar, H**. Amend, C.A, Chen, C., and Benz, C.C. ESX expression during mammary cell transformation or differentiation is determined by a novel epithelial-specific enhancer element and binding complex (*submitted Molecular Cancer Research*).

CONCLUSIONS

The in vivo mammary gland model has made possible the study of normal human breast epithelium and the epithelial-stromal interactions that occur in the breast. The implications of this model are vast since both the epithelium and fibroblast components of the mammary gland can be manipulated to study mammary gland development by using tissue from genetically modified animal models or changes that occur in normal epithelium when grown in association with carcinoma associated fibroblasts

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